and ascorbic acid levels; and whole blood riboflavin levels were drawn. Results: Five of the six patients with normal renal function had low serum ascorbic acid levels (<0.12, < 0.12 mg/dl; normals 0.2–2.0 mg/dl; and 0.4, 0.5, and 0.4 mg/dl; normal 0.6–2.0 mg/dl). Serum riboflavin levels were found to be low in one (1 mcg/L; normal 3–15 mcg/L), but normalized with daily administrtation, and serum retinoids were low in two (each with levels 17 mcg/dl; normal 28–94 mcg/dl). There were no clinically obvious untoward effects due to these vitamin deficiencies. Each of the patients with renal insufficiency had elevated serum pyridoxine levels (49.4 and 33.6; normal 2–29 ng/ml). Each had some neurologic disturbance (peripheral neuropathy, involuntary movements). Following the cessation of the multivitamin preparation, the serum pyridoxine levels fell to normal in each (4.0 and 14.4, respectively), but ascorbic acid levels fell into abnormally low levels. Conclusion, 1. The reduction in the administration of multivitamins from daily to thrice weekly in patients receiving HPN and normal renal function was associated with diminished ascorbic acid levels in 5 of 6 patients. Less often low levels of retinoids and riboflavin are seen. 2. Patients with chronic renal failure receiving HPN with multivitamins may develop elevated pyridoxine levels, which might produce neurologic sequelae.

## G2394

### PROTEIN-LOSING ENTEROPATHY CAUSED BY INTESTINAL LYMPHANGIECTASIA IN A PRETERM INFANT

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Intestinal lymphangiectasia (IL) is characterized by obstruction of lymph drainage from the small intestine and presence of dilated lacteal distorting the villous architecture. There are no reports of IL in premature infants. This male patient was born after a pregnancy of 30 weeks' gestation (birth weight: 1,210 g). His clinical course during the first days of age was characterized by hyaline membrane disease, pneumomediastinum and pneumothorax, intraventricular cerebral hemorrage. He was receiving adequate specific treatment in the intensive neonatal unit care. He was fed with expressed breast milk together with an appropriate formula for preterm infants (Similac 24). By the 7$^{th}$ day of life he showed edema of the legs and head. Biochemical data showed: low serum albumine (2.0, 2.4 g%), low serum IgG (324 mg%) but normal serum IgA (6.2 mg%) and IgM (21.9 mg%); low serum tranferrin and high α-1-antitrypsin stool levels (>3 mg/g dryweight sample). Because of suspected IL an upper endoscopy was performed with a thin upper fiberscope (Olympus, 5.5 mm .). White opaque spots in the duodenum were evident and two bioptic specimens were taken from the areas mostly involved. At the pathologic examination villous architecture was distorted by dilated lymphatic channels; apart from the distorted architecture mucosa was normal, but villous blunting in some areas was noted. Appropriate diagnostic tests had excluded secondary lymphangiectasia (i.e. chronic congestive heart failure, intestinal malrotation). Breast milk and adapted formula were substituted for a formula containing medium chain triglycerides (Portagen, Mead Johnson). At the follow up a significant rise in the serum albumine level was detected (3.1, 3.8 g%) and α-1-antitrypsin stool levels normalized; edema had disappeared and weight gain occurred. We conclude that this case is worthy to be mentioned for several reasons: to our knowledge, it is the first report of IL in a preterm infant; endoscopy is of great value in doing a diagnosis of IL because the latter is a patchy lesion and endoscopy can correctly address the biopsies from the areas of duodenal mucosa involved; finally, upper endoscopy can be successfully performed in very preterm infants.

## ● G2395

### STRUCTURE/FUNCTION STUDIES OF THE INTESTINOTROPIC HORMONE, GLUCAGON-LIKE PEPTIDE-2

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Glucagon-like peptide-2 (GLP-2) is a 33 amino acid gut hormone that stimulates mucosal growth and regeneration in the intestine. To understand the structural determinants of the signal transduction mechanism leading to proliferation, the recently cloned GLP-2 receptor (GLP-2R) was used to analyze the structure/function of GLP-2. To identify functional side-chain groups required for interaction with the GLP-2R, 56 analogs of GLP-2 were assessed with respect to receptor binding and activation. Two types of amino acid substitutions were made in generating the analogs: 1)substitution of the native Ala residue at postion 2 with every other amino acid, and 2)substitution of the native amino acid at positions 1–33 with L-Ala. The Ala scanning peptides were based on a degradation resistant analog of GLP-2, [Gly2]GLP-2, and thus all possessed a Gly at postion 2 as well as their respective Ala mutation. Specific binding and cAMP production were examined using BHK cells stably transfected with the cloned rat GLP-2R. The high and low-affinity $IC_{50}$'s for wild-type GLP-2 were 2.2 ± 9.4pM and 69.1 ± 10.5nM resp., and the $EC_{50}$ was 14.0 ± 2.8nM. The side chains in positions 17,20,22,23,25, 26 and 30 were required for receptor binding. When neutral, hydrophilic amino acids were substituted by Ala at positions 5,11,12,16 and 24, cAMP production was augmented, with an $EC_{50}$ of 1.7 ± 0.5nM for [Gly2,Ala16]GLP-2. Of the Ala2-substituted analogs, only [Pro2] and [D-Ala2] GLP-2 showed increases in cAMP production ($EC_{50}$= 5.1 ± 0.3 and 7.2 ± 0.3nM resp.) as compared to the [Gly2]GLP-2 control ($EC_{50}$= 9.2 ± 0.5nM). These results indicate that single amino acid substitutions within GLP-2 can confer structural changes in the ligand-receptor interface, facilitating the design of more potent analogs for the treatment of patients with compromised intestinal function.

## ● G2396

### SPLANCHNIC GLUTAMINE METABOLISM DURING PREDNISONE-INDUCED PROTEIN WASTING IN HUMANS.

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**Aim and methods**: To determine whether glucocorticosteroid treatment alters splanchnic glutamine (GLN) metabolism, 2 groups of healthy adults received 5-h intravenous infusions of L-[1-$^{14}$C]leucine and L-[$^{2}H_5$]glutamine, along with *q.* 20-min oral sips of tracer doses of L-[1-$^{13}$C]glutamine in the fasting state, either: **(a)** at baseline (control group; n=6); or **(b)** after a 6-d course of 0.8 mg.kg$^{-1}$.d$^{-1}$ prednisone (prednisone group; n=8). Leucine appearance rate (Ra) and oxidation were determined from plasma $^{14}$C-keto-isocaproate, and breath $^{14}CO_2$, respectively. GLN appearance rate (Ra*gln*) was determined from plasma $^{2}H_5$-GLN enrichments; GLN release from protein breakdown (B*gln*) was extrapolated from leucine Ra, an index of proteolysis; GLN *de novo* synthesis (DS*gln*) was estimated by DS*gln*=Ra*gln*-B*gln*. Splanchnic glutamine uptake (SU*gln*) was estimated by the fraction of orally administered $^{13}$C-glutamine that failed to appear into systemic blood; and glutamine oxidation, from breath $^{13}CO_2$ excretion. **Results**: As expected, prednisone treatment increased leucine Ra, an index of proteolysis, and leucine oxidation (134±8 vs 160±4, and 23±2 vs 35±7μmol.kg$^{-1}$.h$^{-1}$, respectively, both p<0.05), and plasma GLN concentration (565±9 vs 643±6μmol/L; p<0.05). **Conclusions:** Prednisone-induced protein wasting is associated with 1) markedly enhanced GLN *de novo* synthesis (+59%), slightly (NS)increased GLN oxidation; and 2) a dramatic (+52%) rise in splanchnic GLN utilization. *Supported by NIH grant RO1-DK51477.*

| μmol.kg$^{-1}$.h$^{-1}$ | Ra*gln* | DS*gln* | OX*gln* | SU*gln* |
|---|---|---|---|---|
| Control | 301±41 | 248±44 | 77±4% | 42±4% |
| Prednisone | 475±24* | 394±33* | 87±9%* | 64±6%* |

*p<0.05

## G2397

### EFFECT OF SOLUBLE NON-FERMENTABLE FIBER AND WHEAT BRAN ON ABERRANT CRYPT FOCI (ACF) IN A CHEMICALLY INDUCED RAT MODEL OF CARCINOGENESIS

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To further elucidate possible mechanisms of colon cancer risk reduction by dietary fiber, we compared psyllium (PS), a partially fermentable viscous fiber, to methylcellulose (MC), a nonfermentable viscous fiber, in combinations with wheat bran (WB), in an animal model of colon cancer. **Methods**: Male Fisher 344 rats were randomized into 7 groups of 10 animals each and fed for 26 weeks diets containing one of the following fiber sources (8% w/w) in a semi-purified dietary base: WB, microcrystalline cellulose (C), MC, WB:PS (1:1), or WB:MC (1:3,1:1, and 3:1). The rats were injected with azoxymethane, 15 mg/kg s.c., on weeks 5 and 6. Rats were killed by $CO_2$ inhalation, and colon, liver, and blood samples were obtained. ACFs were quantitated from methylene blue stained, formalin fixed sections by a blinded observer. **Results**: As expected, inclusion of PS increased colon weight; MC did not. The proportion of large *vs.* small ACFs did not vary by colon segment or diet. The WB diet showed the lowest number of ACFs (78±7, mean±SE), similar to C (87±8), while the WB:PS and 3:1 WB:MC diets were significantly higher (133±9 and 106±9, resp., p<0.05). MC alone was also significantly higher (119±11) *vs.* WB; however, 1:1 and 1:3 mixtures of WB and MC were as effective as WB alone. All three combinations of WB with MC yielded significantly fewer ACFs than the WB:PS diet. Labeling indices and fecal composition will be reported. **Conclusions**: It has been hypothesized that fermentation products of soluble fiber, particularly butyrate, are responsible for its protective effect. Our results suggest that fermentation alone cannot account for reduced ACF formation. Sustained viscosity and/or water holding capacity may be critical properties of a soluble fiber with respect to chemoprotection.

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# Stability of Ingested Methylcellulose in the Rat Determined by Polymer Molar Mass Measurements by Light Scattering

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Methylcellulose (MC) is ingested by humans in food and pharmaceutical formulations. The functional properties of MC like those of other linear polymers depend primarily on polymer length or molar mass for largely linear polymers. Although many studies in animals and humans have shown complete excretion of MC, in vitro human fecal fermentation studies indicate that MC can be degraded and presumably lose some of its functionality. In this study, MC polymer distribution in the feces from rats fed a diet containing 8% methylcellulose were compared to the fed MC. The water-soluble polymers in the feces were separated by a size exclusion chromatography (SEC) and the polymer distributions determined by multiple angle laser light scattering (MALLS). Detection of the fluorescent MC−calcofluor complex was used to confirm the identity of the eluting MC peak. All dietary MC was recovered in the feces. There is a small shift ($P < 0.06$) in the weight-averaged molecular weight of polymer distribution of MC extracted from the feces to $2.71 \pm 0.15 \times 10^5$ g/mol from $3.15 \pm 0.02 \times 10^5$ g/mol in the standard. There is also an increase in the polydispersity from 1.21 in the standard to 1.8 in the fecal extract. The distribution of the substituted methoxylated glucose monomers by gas chromatography also confirms the stability of MC fed to rats. The amount of actual hydrolysis is estimated to be about 0.1 glycosidic linkage/molecule. MC is not easily determined by standard dietary fiber methods, and SEC with MALLS and/or fluorescence may be a useful alternative.

**KEYWORDS: Methylcellulose; rat; polymer distribution; light scattering; enzymatic hydrolysis; liquid chromatography; gas chromatography; size exclusion**

## INTRODUCTION

Methylcellulose (MC) has been widely used by the food, cosmetics, and pharmaceutical industries since it was first produced in 1938. Methylcellulose is synthesized by heating cellulose fibers with methyl chloride under alkaline conditions to form methyl ethers of the free hydroxyl groups (*1*) of the glucose subunits that form the backbone of the cellulose polymer. The resulting polymer has the interesting property of being cold but not hot water soluble and forms useful, viscous solutions even at very low concentrations. Since humans ingest MC its toxicology in short and long-term animal feeding studies has been reviewed with the conclusion that consumption has no adverse effects (*2*). Human subjects fed MC excreted 65 to 100% of the ingested polymer (*3*). Most of the excreted MC was inferred to be of high molecular weight since it was precipitated by hot water. The MC in the fraction not precipitated by hot water was thought to be low molecular weight polymers formed by the degradation of MC polymer into smaller fragments by colonic bacteria. The stability of MC to digestion or fermentation by colonic bacteria to absorbable hydrolysates is substantiated by the finding that rats fed $^{14}$C-labeled MC do not excrete $^{14}$C-labeled methanol or its metabolites in the urine nor is radioactivity found in the tissues (*4*). Although MC is very stable in the digestive tract, Machle et al. (*3*) demonstrated that incubation of MC with human feces for 2 days resulted in about 30% lower recovery. More recently, the incubation of human colonic bacteria with MC of viscosity grades 15, 1500, and 4000 from 4 to 24 h resulted in significant decrease of alcohol precipitated organic matter but without the evolution of methane, other volatile gases, or short chain fatty acids (*5*). These studies indicate that the methyl groups of MC in vivo or in vitro are not metabolized and used as a source of energy, but that under certain circumstances the polymer can be enzymatically hydrolyzed to polymers of lower molecular weight that may be difficult to recover by precipitation by either alcohol or hot water.

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Since the physical and physiological properties of MC depend primarily on polymer size, determination of the molecular mass distribution of the polymer is a straightforward means of determining the degree of degradation of MC in the digestive tract. However, MC is difficult to recover from complex matrixes such as food and feces, and apparently becomes more difficult when the polymer is degraded and solubility increases. Most MC recovery analysis was conducted by the methodology available at the time, gravimetric or gas chromatographic methods, that do not reveal polymer size distribution characteristics. In recent years, high performance size-exclusion liquid chromatography (HPSEC) has been used to separate polymer mixtures and determine their molecular mass distribution. The high aqueous solubility of MC at room temperature becomes advantageous when using HPSEC. By using a system of detectors in conjunction with HPSEC that simultaneously identifies soluble cellulosic polymers, determines molecular weight of the polymer, and determines mass of eluting solute, we were able to determine the amount and characteristics of MC in feces from rats fed MC. Gas chromatography was also used to independently confirm that MC was largely excreted unchanged in the feces by comparing the experimental to the expected distribution of the tri-, di-, mono-, and unsubstituted glucose units of MC in the feces.

## MATERIALS AND METHODS

**Animal Care and Handling.** Male Fischer 344 rats, 5 weeks after birth, were purchased from Simonsen Laboratories, Gilroy, CA. After two weeks on chow, the animals were randomly assigned to the six treatment groups, 10 animals per group, and fed the experimental diets for 26 weeks. Animals were weighed once each week. Diets were prepared monthly and fed twice each week. Feces were collected for 5 days during the 24th week. Animals were housed individually with free access to feed. Lighting in the room was maintained on a reverse 12 h (light 5 PM−5 AM) cycle. All animal procedures were approved by the Animal Care and Use Committee of the Western Regional Research Center, USDA, Albany, CA, and conform to the principles in *Guide for the Care and Use of Laboratory Animals* (*6*).

The diets contained 8% total dietary fiber (TDF) made up of MC or cellulose fiber, 81.6 g/kg diet, or combinations of wheat bran and methylcellulose in varying ratios to evaluate matrix effects. The remainder of the diet contained (per kg diet): wheat gluten, 44.6 g; dextrose, 234.1 g; corn oil, 92.4 g; casein, 163.4 g; sucrose, 228.9 g; lard, 88.4 g; hydrogenated coconut oil, 19.2 g; dl-methionine, 2.0 g; choline bitartrate, 2.0 g; vitamin mix, 10.0 g; and mineral mix, 35.0 g. Ingredients were purchased from Dyets (Bethlehem, PA) except the Dow MC (30.9% methoxyl substituted and apparent viscosity of 4894 cps) that was donated by GlaxoSmithKline (Philadelphia, PA).

Polymer analysis: Solutions for size-exclusion chromatography were prepared by treating feces or MC standards (50 mg) with ethanol (0.5 mL) for 5 min, adding 0.02% sodium azide (4.5 mL), gently mixing (rocking action) overnight at 23 °C, and filtering through a 0.45 $\mu$m nylon #2359 filter (Alltech Associates, Inc., Deerfield, IL). The polymer components in these solutions (100 $\mu$L) were separated by HPSEC. The system included control software, pumps (model 1100, mobile phase and Calcofluor reagent), refractive index detector (model 1100), and fluorescence detector (model 1046) from Agilent (Palo Alto, CA), Aquagel columns (3 linear OH + OH-60, Polymer Laboratories, Amherst, MA), and a multiple angle laser light scattering detector (MALLS, Dawn DSP-F, Wyatt Technologies, Santa Barbara, CA). Calcofluor White (CAS 4404-43-7) was purchased from Sigma, St. Louis, MO. The polymer components were separated by elution with 0.02% sodium azide (0.6 mL/min). Positive identification of the MC as the calcofluor complex was achieved by simultaneous fluorometric detection (Ex = 415 nm, Em = 445 nm) upon introduction of calcofluor reagent (0.1 mg/L 0.1 N NaOH, 0.7 mL/min) into eluant stream by a mixing-T following the RI detector. Polymer mass calculated from RI and light scattering data was fitted (first degree, Zimm equation (Kc/R(q)) (*7*) to a line whose intercept is the molecular weight (g/mol) of the polymer. Peak characteristics were compared using Student's t-test.

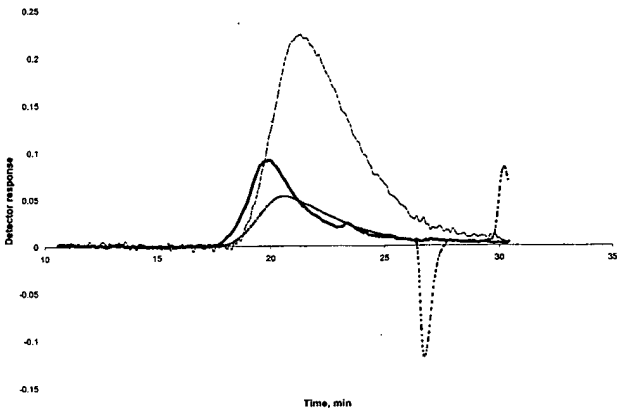


**Figure 1.** Chromatographic detection of methylcellulose after size-exclusion chromatography. Traces are laser light scattering detector (heavy line), refractive index (medium line), and fluorescence detector after simultaneous postcolumn reaction with calcofluor (thin line).

**Methylated Glucose Analysis.** Fecal carbohydrate monosaccharide composition was determined by flame ionization on an Agilent 5890 gas chromatograph (Palo Alto, CA). Samples were prepared according to Olson et al. (*8*). Briefly, 10−20 mg of ground feces were hydrolyzed with 0.1 N trifluoroacetic acid followed by reduction by sodium borohydride at 25 °C for 1 h. The resulting alditol was acetylated with acetic anhydride/pyridine at 121 °C, 2 h. The alditol acetates were separated on an Agilent (model 5890, Wilmington, DE) gas chromatograph fitted with a 30 m nonpolar capillary column (#DB-5MS, J&W Inc, Sacramento, CA) and quantitated by FID detection.

## RESULTS

The molar mass distributions of the standard MC and fecal MC samples were determined by analysis of the data collected from the multiangle laser light scattering (MALLS) and refractive index (RI) detectors after separation of the polymer size exclusion chromatography (SEC). The molar mass is a function of the intensity and angular dependence of the scattered light and the intensity of the RI signal. The polymers in each of the 400 peak segments are assumed to be homogeneous and thus the Zimm equation can be applied. The elution time and chromatographic characteristics of the MC standard were determined first and are shown in **Figure 1**. Standard MC polymer elutes between 17 and 27 min and its elution is detected simultaneously by RI, 90° angle of the MALLS, and fluorescence (FL) after postcolumn derivatization with calcofluor. Calcofluor is specific for polysaccharides with contiguous (1 → 4)-$\beta$-linked D-glucopyranosyl units. The weight averaged molar mass over the entire peak, Mw, was $3.154 \pm 0.02 \times 10^5$ g/mol, and polydispersity (Pd) was $1.21 \pm 0.02$. Polydispersity is the ratio of Mw and Mn, number average molar mass.

The differential molar mass distributions of standard MC (triplicate, heavy lines) and MC fed to and extracted from the feces of rats (10 animals, light lines) fed MC as the only source of dietary fiber are shown in **Figure 2**. There is a small shift ($P < 0.06$) in the polymer distribution of MC extracted from the feces from $3.15 \pm 0.02 \times 10^5$ g/mol in the standard to $2.71 \pm 0.15 \times 10^5$ g/mol. There is a significant shift ($P < 0.01$) in the peak molar mass from $3.07 \pm 0.18 \times 10^5$ to $2.60 \pm 0.22 \times 10^5$ g/mol of the standard and feces, respectively. There may be a small bias to lower molecular weight in the fecal sample due to the small peak appearing between 40 and 80 000 g/mol. This peak is probably not derived from MC since there is no

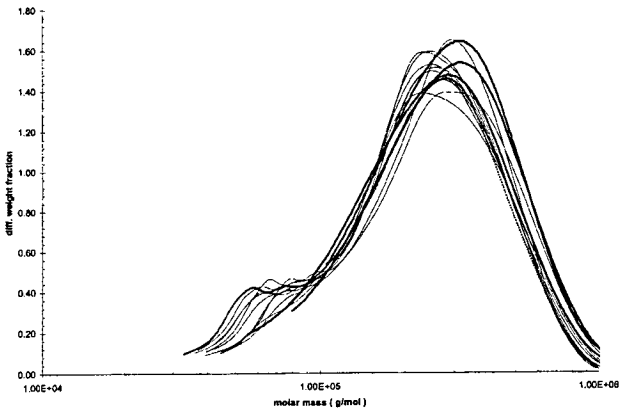


**Figure 2.** Differential Mw distribution of methylcellulose from feces (light lines) of 10 rats compared to standard methylcellulose (heavy lines, triplicate) in feed.

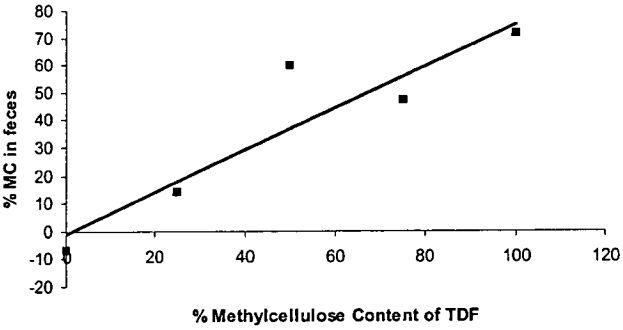


**Figure 3.** Percentage of methylcellulose in feces of rats fed 8% dietary fiber consisting of 0, 25, 50, 75, and 100% methylcellulose with balance made up with wheat bran fiber.

calcofluor fluorescent peak eluting after the main MC peak in the chromatogram. The Pd was also greater for the MC from the feces, 1.83 ± 0.16, compared to the standard, 1.21 ± 0.02.

To evaluate matrix effects on MC determinations, the MC content was measured in feces from the rats fed the diets with varying ratios of MC and wheat bran. These diets contained 0, 2, 4, 6, or 8% MC, and the balance of the 8% TDF was made up by dietary fiber from wheat bran. The area under the RI signal was used to determine eluted MC mass in the fecal extract. The small negative value of the feces with no MC is due to the integration of a small part of the refractive index trace that becomes negative when the water used to extract the feces elutes. The mass of MC in the fecal extract is directly related to the amount of MC (R2 = 0.842) in the diet (**Figure 3**). The linear relationship indicates that MC can be accurately determined even in matrixes with large amounts of other sources of TDF.

In addition to the polymer distribution and dose response to fed MC, we compared the amount of MC determined by SEC and RI detection with the amount predicted from consumption data. A standard curve (R2 = 1) relating the area under the RI detector signal (mass MC eluted, g) to standard MC solution concentrations (0.5−4 mg/mL) was used to quantitate the amounts of MC in feces. The feces from two rats were collected for 5 days and averaged 1.39 and 1.86 g/d. The MC content of the feces based on HPLC analysis was 1.0 and 1.4 g/d, respectively. The feed intake of the two rats fed a diet containing 8% MC over a seven day period averaged 12.5 and 14.7 g of feed/day, respectively. The daily MC intake was 1.0 and 1.2 g, respectively, and in good agreement with the recovery data by

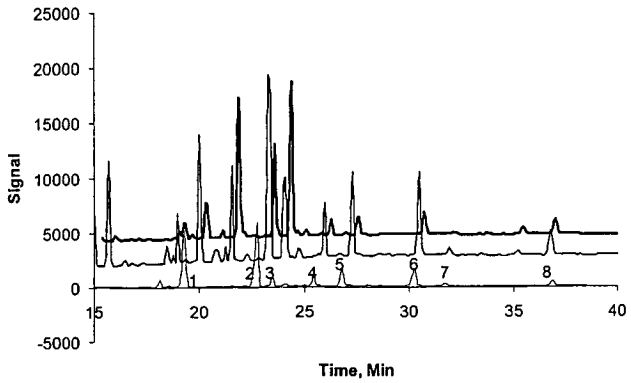


**Figure 4.** Gas chromatographic traces of acetylated methylglucitols prepared from standard methylcellulose (bottom), rat fed 75% MC and 25% wheat bran fiber (middle) and rat fed 25 and 75% wheat bran fiber (top). The peaks are identified in the standard (bottom trace) as 2,3,6-trimethyl- = 1; 2,6-dimethyl- = 2; 3,6-dimethyl- = 3; 2,3-dimethyl- = 4; 6-monomethyl- = 5; 2-monomethyl- = 6; and 3-monomethylglucitol = 7. Peak 8 is glucitol.

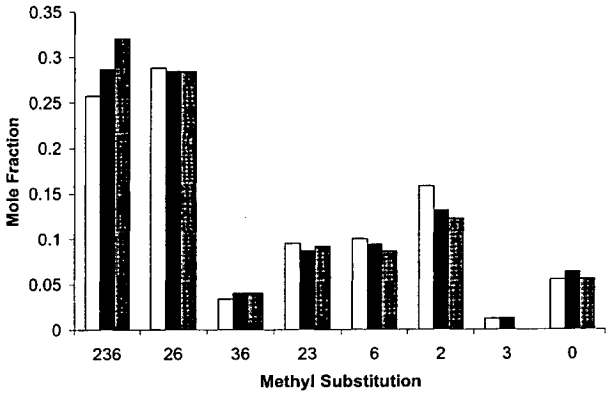


**Figure 5.** Mole fraction composition of methylated glucose components of methylcellulose from feces of rat fed methylcellulose (gray bar) compared to standard methylcellulose (black bar) and published composition (white bar) (*11*).

SEC. The amounts of MC consumed and excreted support the polymer profile of MC in the feces and agree with reports of lack of absorption of MC in previous studies without direct determination of the polymer.

MC is often quantitated by gas chromatography as the acetylated alditol after acid hydrolysis, sodium borohydride reduction, and esterification with acetic anhydride (*9*−*11*). This method is very useful but cannot definitely characterize the polymer distribution. The gas chromatogram of the derivatized MC hydrolysates is very characteristic (**Figure 4**) and the peaks are easily identified even in chromatograms from fecal samples. The most prominent peak is usually the acetylated 2,6-dimethylglucitol followed by the acetylated 2,3,6-trimethylated glucitol. Together they make up about 50 mole percent of the eight possible glucose products. The other products are the 3,6- and 2,3-dimethylglucitol and the 2-, 6-, and 3-monomethyl glucitol derivatives as well as the completely unsubstituted glucitol. The 3-position is about 10% less substituted in the 2,3-dimethyl and 3-methylglucitol compared to the other di- or monomethyl substituted glucitols, respectively, as shown in **Figure 5** and **Table 1**. The mole fractions of the eight methylated glucitols from the feces are similar to the ratios in the standard and to those reported in the literature (*11*).

**Table 1.** Molar Ratios of Alditol Acetates from Methylcellulose Hydrolysates of Standard, Rat Fecal Extract, and Published Ratio[a]

| RT, min | Me subsitution | molar ratio stnd | molar ratio rat fecal extract | molar ratio MC-3[a] |
|---|---|---|---|---|
| 20.86 | 2,3,6 | 0.287 | 0.321 | 0.257 |
| 24.29 | 2,6 | 0.285 | 0.284 | 0.288 |
| 25.01 | 3,6 | 0.040 | 0.040 | 0.034 |
| 26.97 | 2,3 | 0.087 | 0.092 | 0.095 |
| 28.83 | 6 | 0.094 | 0.086 | 0.100 |
| 31.77 | 2 | 0.131 | 0.122 | 0.158 |
| 33.19 | 3 | 0.013 | 0.000 | 0.012 |
| 38.38 | 0 | 0.063 | 0.056 | 0.055 |
| total | | 1 | 1.001 | 0.999 |

[a] Ref 9.

## DISCUSSION

Previous recovery studies of MC after passage through the digestive system of humans or animals have not directly determined the molecular size of the polymer. Yet, the physical, rheological, and most probably the physiological properties of aqueous MC solutions are highly dependent on the chain length of the cellulose polymer (*9*, *12*). Size exclusion chromatography was able to separate MC from other polymers in feces so that light scattering methods could be used to determine the molar mass of the eluting polymer. The MC peak was identified by its retention time and the formation of a fluorescence complex with calcofluor. Methylcellulose and other cellulose derivatives, $\beta$-glucans, and some cereal pentosans are food sources of polymers that form calcofluor complexes. The column set in this study was able to separate MC in the presence of high levels of wheat bran cellulose and hemicellulose in the feces as shown by the linear recovery of MC from feces of rats fed diets containing various levels of wheat bran and MC.

The differential molecular weight distributions of MC from the fecal extracts of rats fed only MC as the sole source of fiber shows some hydrolysis has occurred. There is a shift of average Mw over the entire peak ($P < 0.06$) and maximum Mw of the peak ($P < 0.01$) to lower values, $2.82 \pm 0.18 \times 10^5$ (mean $\pm$ SD) and $2.60 \pm 0.22 \times 10^5$ g/mol, respectively, from the standard's peak average, $3.15 \pm 0.02 \times 10^5$ g/mol, and the peak maximum, $3.07 \pm 0.18 \times 10^5$ g/mol. There is also an increase in the Pd from 1.21 in the standard to 1.8 in the fecal extract. Viscometric methods have reported similar values for Mw but higher values of about 4 for Pd (*10*). However, similar Pd values, 1.18−1.92, were reported for hydroxypropylmethyl celluloses when MALLS detectors were used (*13*).

The decrease in Mw and increase in Pd are characteristic of enzymatic hydrolysis. The enzymatic degradation of guar by a random hydrolysis, single scission pathway resulted in decreased Mw and increased Pd. Sonication of guar or homogenization of MC decreases polymer Mw and Pd (*13*, *14*). However, the actual number of glycosidic linkages hydrolyzed is probably very small. If the scission number, $S$, defined as $M(0)/M(t) - 1$, is calculated using the values for the standard and fecal Mw for M(0) and M(t), respectively, $S$ will equal 0.08. If each polymer were randomly cut once, the Mw of the resulting mixture would be one-half the original and the scission number would be 1. In this case, approximately one glycosidic bond is hydrolyzed in every 10 polymer molecules.

Enzymatic hydrolysis is probably slow because the methylated cellulose polymer is not a good substrate for cellulases. The substitution pattern of methylated glucose units has been shown to be random (*11*), and there exist infrequent regions where three adjacent glucose units are not methylated. Enzymatic hydrolysis at these uncommon sites is possible and results in a small number of bond scissions that would lead to polymers of significantly decreased chain length and viscosity. Cellulases usually prefer glycosidic linkages adjacent to unsubstituted glucose and then 6-*O*-methylated glucose (*10*). Together these sites make up about 15% of the polymer (**Table 1**). This suggests that although the cleavage sites are adjacent to 6-*O*- or unsubstituted glucose units, contiguous unsubstituted or monosubstituted glucose units are probably required for observable enzymatic activity. The presence of three contiguous unsubstituted glucose units in MC has been calculated and experimentally demonstrated to be less than 0.01 mole fraction (*11*). An average sized MC polymer molecule would contain about 500 trimer segments and therefore at most five contiguous unsubstituted glucose units per average molecule. The low rate of observed hydrolysis suggests that more than three unsubstituted or monosubstituted units are required for ready hydrolysis during the digestive time period and that differences in experimental results might be due to differences in degrees of methoxylation as well as recovery methodology.

These results suggest that fecal enzymes in vitro can hydrolyze MC but that the hydrolysis in vivo is very slow presumably due to the lack of contiguously unsubstituted glucose units. The fermentation of MC compared to psyllium, pectin, and Solka Floc cellulose, in an in vitro system using human fecal inoculates, resulted in 29.1−79.0% disappearance of organic matter (OMD) from the MC substrate in 4 h (*5*). However, short chain fatty acid production and total gas production ($CH_4$, $CO_2$, $H_2$) was almost nonexistent compared to pectin and psyllium, and about the same level as Solka Floc. The latter is a cellulose fiber that was almost completely recovered. Fermentation of MC to cell mass rather than gas or SCFA was suggested as the fate of the OMD. Methylcellulose was recovered by precipitation in a solution finally containing 76% alcohol. Methylcellulose is difficult to recover by alcohol precipitation, and this difficulty may be the cause of the discrepancy between high OMD but negligible fermentation metabolites. Another possibility is polymer hydrolysis to polymers of lower Mw and increased aqueous alcohol solubility resulting in lower recovery but also lack of fermentation products. Since the rats in this study were fed diets containing 8% MC as the only fiber source continuously for a period of 24 weeks, it is expected that the colonic microflora had adapted as much as possible to utilize MC as an energy substrate. The experimental conditions of in vitro conditions may contribute more to MC degradation than occurs in mammalian digestive systems.

Although polymer size information is not obtained directly from gas chromatographic data the pattern of methylated glucose monomers is consistent with the excretion of an undigested polymer. The methylated glucose components of MC (2,3,6-trimethyl; 2,6-, 3,6-, and 2,3-dimethyl; and the 2-, 3-, and 6-methylglucose) are easily identified in the acid hydrolyzed fecal samples as the borohydride reduced and acetylated alditols (**Figure 4**). The most highly methylated glucitol elutes first followed by the less methylated (and more acetylated) glucitols. The alditols derived from MC are easily distinguished from other dietary fiber derived monosaccharides even when fed in combination with other sources of fiber such as cereal bran (data not shown).

The degree of methyl substitution and the distribution of the tri-, di-, mono-, and unsubstituted glucose components are key

to the potential for enzymatic hydrolysis. The distribution of the methylated glucose monomers of MC in this study is similar to the results reported by Arisz et al. (*11*) who also determined monomers by gas chromatography as their acetylated alditols (**Figure 5**). These authors calculated the distribution of methylated derivatives and found that nonsubstituted and trimethylglucosyl units were underestimated, suggesting that some regions of MC were densely derivatized and other regions were poorly derivatized. Analysis of the perdeuteriomethylated glucose trimers from partially hydrolyzed MC by fast atom bombardment mass spectrometry showed that a small fraction, 3−6%, was not methylated at all. These are potential sites for enzyme attachment and hydrolysis.

An indirect measure of sparse methylation is also evident in the report by Wood (*15*), who showed that dye binding by cello-oligosaccharides required at least five residues. Wood also reported that hydroxyethyl but not carboxyethylcellulose could bind congo red or calcofluor indicating that some substituted celluloses have regions of five or more continuously unsubstituted glucose units. Engle et al. (*16*) reported the binding of congo red to MC and found that its circular dichroic spectra were between that of cellotetraose and cellopentaose. Hydroxypropylcellulose bound congo red more strongly than cellopentaose. This supports Wood's conclusion that five continuously unsubstituted glucose units are necessary for dye binding. These studies indicate that regions of low substitution occur in MC and other substituted cellulose polymers, and are the most probable sites when enzymatic hydrolysis occurs. The interaction of MC with cellulases is variable and MC has been shown to inhibit cellulases of several ruminal bacteria (*17*).

The MC recovery from feces as detemined by HPLC accounts for about 100 and 117% of the MC in the diet consumed by two rats fed MC as the only source of an 8% TDF diet. This observation by SEC and calcofluor complex formation is supported by previous reports of the apparently complete recovery from the feces by gravimetric methods and the lack of radioactivity in tissues and urine using $^{14}C$-labeled methoxyl groups.

Methylcellulose in food is a form of soluble dietary fiber, but its determination by standard methods is at best incomplete (*18*). Methylcellulose is not precipitated by alcohol, as are most other soluble dietary fibers. Although the formation of fluorescent complexes with substituted celluloses is well-known, it does not appear that the combination of size exclusion chromatography and calcofluor complex formation has been used to determine MC in biological matrices. Wood and Fulcher (*19*) reported that calcofluor precipitated the $\beta$-1,4-glucose based polymer, hydroxyethylcellulose, but not the $\beta$-1,4-glucose polymers: cellobiose, laminaran, or carboxymethylcellulose.

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